Fate of T-2/HT-2 toxins during durum wheat processing

Occurrence of T-2/HT-2 toxins in durum wheat and pasta
The European semolina industry in a few figures

The European semolina industry implies:

- 231 production units:
  - 162 production units in Italy;
  - 16 production units in Greece;
  - 8 production units in Spain;
  - 7 production units in France;
  - 5 production units in Germany.

- 7,300,000 tons of durum wheat transformed:
  - 5,100,000 t in Italy;
  - 675,000 t in France;
  - 550,000 t in Spain.

- 4,850,000 tons of durum wheat semolina produced.

Union of Associations of Semolina Producers of the EU Countries

The Union of Associations of Semolina Producers of the EU countries has the purpose of ensuring the representation and promotion of the interests of the semolina industry of the European Union at European and International levels.
**Durum Wheat: a rare and precious cereal**

With an annual production, calculated on the basis of the last ten years, of approximately 35 Mt, durum wheat represents, at world level, only a «secondary» cereal, as regards quantity.

In the EU, and limited to the years subsequent to the Reform of the CAP Agenda 2000, this production has amounted to between 7.1 and 11.1 Mt, for an average annual production of 8.4 Mt.

- **An extremely localised world production**
  The Mediterranean basin and the North American continent alone represent almost 80% of the world production of durum wheat;

- **A quantitatively fluctuating world production**
  The agronomic situation and the particular climatic conditions of the areas producing durum wheat lead to an extreme variability in production which may take the form, year by year, of quantitative fluctuations in the order of 35%.
The production of Durum Wheat in the EU

The EU production of durum wheat is not able to cover the quantitative requirements, but neither the qualitative requirements, of the semolina industry of the EU.

<table>
<thead>
<tr>
<th>Year</th>
<th>Production (Mt)</th>
<th>Imports (Mt)</th>
<th>Import/Production (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>8.8</td>
<td>0.9</td>
<td>10.2%</td>
</tr>
<tr>
<td>2001</td>
<td>7.3</td>
<td>1.5</td>
<td>20.6%</td>
</tr>
<tr>
<td>2002</td>
<td>9.2</td>
<td>0.6</td>
<td>6.5%</td>
</tr>
<tr>
<td>2003</td>
<td>7.9</td>
<td>2.0</td>
<td>25.3%</td>
</tr>
<tr>
<td>2004</td>
<td>11.1</td>
<td>1.6</td>
<td>14.4%</td>
</tr>
<tr>
<td>2005</td>
<td>7.1</td>
<td>1.6</td>
<td>22.5%</td>
</tr>
<tr>
<td>2006</td>
<td>7.8</td>
<td>1.6</td>
<td>20.5%</td>
</tr>
<tr>
<td>2007</td>
<td>7.1</td>
<td>1.9</td>
<td>26.7%</td>
</tr>
<tr>
<td>2008</td>
<td>9.9</td>
<td>1.4</td>
<td>14.1%</td>
</tr>
<tr>
<td>2009 (estimates)</td>
<td>7.9</td>
<td>1.7</td>
<td>21.5%</td>
</tr>
</tbody>
</table>

Quantity of durum wheat transformed 7,300,000 t
Production of semolina 4,850,000 t
Intake estimates indicate that the presence of T-2 and HT-2 toxin can be of concern for public health. Therefore, the development of a reliable and sensitive method, collection of more occurrence data and more investigations/research in the factors involved in the presence of T-2 and HT-2 toxin in cereals and cereal products, in particular in oats and oat products, is necessary and of high priority.

**T-2 and HT-2 toxins**

Provisional Maximum Tolerable Daily Intake

\[ \text{PMTDI} = 60 \text{ ng / kg body weight / day} \]

LOEL (lowest-observed effect level) = 29 µg/kg of body weight per day (pigs) - Safety factor = 500
Fate of T2/HT2 toxins during durum wheat processing
Determination of T-2 and HT-2 toxins in durum wheat and milling fractions

**Extraction**
(with methanol:water + NaCl)

- Filtration (Whatman Nº 4)
- Dilution with water (1:5)
- Filtration (Whatman GF/A)

**Immunoaffinity column clean-up**
(T-2 Test™ HPLC)

**LC-MS/MS determination**
Multiple Reaction Monitoring (MRM) mode
(5 transitions monitored per toxin, internal standard used)

$^{13}$C$_{24}$T2 toxin and $^{13}$C$_{24}$HT2 toxin (Biopure, Austria)
## Recoveries and Limit of detections (LOD)

<table>
<thead>
<tr>
<th>Milling fraction</th>
<th>Recovery (CV)* (%)</th>
<th>LOD (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-2</td>
<td>HT-2</td>
</tr>
<tr>
<td>wheat kernels</td>
<td>104 (3)</td>
<td>107 (2)</td>
</tr>
<tr>
<td>fine middlings</td>
<td>92 (5)</td>
<td>85 (5)</td>
</tr>
<tr>
<td>semolina</td>
<td>102 (3)</td>
<td>101 (5)</td>
</tr>
<tr>
<td>pasta</td>
<td>101 (10)</td>
<td>102 (2)</td>
</tr>
<tr>
<td>screenings</td>
<td>98 (3)</td>
<td>85 (6)</td>
</tr>
<tr>
<td>bran</td>
<td>90 (5)</td>
<td>92 (9)</td>
</tr>
<tr>
<td>red dog</td>
<td>103 (14)</td>
<td>91 (7)</td>
</tr>
</tbody>
</table>

*spiking levels: 100 ng/g T-2 and 100 ng/g HT-2 (n= 3)
**Durum wheat samples origin**

*Inoculated test plots*

**Field trials:** 2007-2008 and 2008-2009 growing seasons (Northern and Southern Italy).

**Fungal inoculum:** *Fusarium sporotrichioides (ITEM 707)* from the collection of the CNR-ISPA (www.ispa.cnr.it/Collection).

**Wheat variety:** Levante (*Triticum durum* Desf.).

**Experimental design:** randomised blocks with 4 replicate plots per trial (plot area 20 m²). Treatments with three different fungicides (prochloraz, tebuconazole, prothioconazole), one *Fusarium* inoculated control and one non-inoculated control.

**Fungicide treatments:** within 24 hours before fungal inoculation (beginning of anthesis, BBCH 61).
Processed samples
Uncleaned durum wheat

✓ Harvest 2008 – 4 level of contamination:

\[ T-2 + HT-2 \ (ng/g) \quad 100 - 235 - 450 - 590 \]

✓ Harvest 2009 – 7 level of contamination:

\[ T-2 + HT-2 \ (ng/g) \quad 221 - 507 - 640 - 720 - 1160 \\
1440 - 5954 \]
Pilot process flow

**Cleaning**
Rationel Kornservice Mod. M220V
(5 mm × 15 mm e 2 mm × 19 mm)

**Uncleaned wheat**

**Screenings**

**Cleaned Wheat**

- **Bran**
- **Red dog**
- **Fine middlings**
- **Semolina**

**Tempering**
(final moisture 17%, 14 h + 3 h)

**Milling**
Bühler Mill Model MLU 202

**Pasta production**
Sercom extruder + Braibanti dryer

**Pasta**
Levels of T-2 + HT-2 in durum wheat milling fractions*

<table>
<thead>
<tr>
<th>Fraction</th>
<th>T-2 + HT-2 (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncleaned wheat</td>
<td>221 ± 10 ng/g</td>
</tr>
<tr>
<td>Cleaned wheat</td>
<td>68</td>
</tr>
<tr>
<td>Bran</td>
<td>317</td>
</tr>
<tr>
<td>Red dog</td>
<td>245</td>
</tr>
<tr>
<td>Fine middlings</td>
<td>46</td>
</tr>
<tr>
<td>Semolina</td>
<td>23</td>
</tr>
<tr>
<td>Pasta</td>
<td>2</td>
</tr>
</tbody>
</table>

Screenings: 834 ± 80 ng/g

T-2 + HT-2 = 221 ± 10 ng/g

* mean ± SD (n = 4)
Levels of T-2 + HT-2 in durum wheat milling fractions*

T-2 + HT-2 = 507 ± 29 ng/g

* mean ± SD (n = 4) from inoculated test plots
Distribution of T-2 + HT-2 (%) in milling fractions*

* mean ± SD (n = 11) from processing of durum wheat contaminated with T-2 + HT2 in the range 97-5954 ng/g (four replicated experiments)
**Distribution of T-2 (%) in milling fractions**

![Bar chart showing the distribution of T-2 in various milling fractions.](image)

- Uncleaned wheat: 100% ± SD (n = 11)
- Cleaned wheat: 41%
- Bran: 160%
- Red dog: 138%
- Fine middlings: 24%
- Semolina: 12%
- Pasta: 1%

**Screenings = 86.8%**

*mean ± SD (n = 11) from processing of durum wheat contaminated with T-2 in the range 35-785 ng/g (four replicated experiments)*
Distribution of HT-2 (%) in milling fractions*

* mean ± SD (n = 11) from processing of durum wheat contaminated with HT-2 in the range 62-5169 ng/g (four replicated experiments)
CONCLUSIONS

An important level of T-2 + HT-2 reduction occurs during the different steps of durum wheat processing relative to the uncleaned wheat:

- ✔ 52% in cleaned wheat
- ✔ 88% in semolina
- ✔ 99% in pasta

T-2 and HT-2 increase significantly in the screening and bran fractions relatively to uncleaned wheat:

- ✔ ~ 600% in screenings
- ✔ ~ 300% in bran
Occurrence of T-2/HT-2 toxins in durum wheat and pasta
Determination of T-2 and HT-2 toxins in wheat and pasta

Extraction
(with methanol:water + NaCl)

Filtration (Whatman Nº 4)
Dilution with water (1:5)
Filtration (Whatman GF/A)

Immunoaffinity column clean-up
(T-2 Test™ HPLC)

Pre-column derivatization with 1-AN
HPLC/FD determination

λ_{ex.} = 381 nm
λ_{em.} = 470 nm

Visconti et al. (2005), J. Chromatography A, 1075, 151-158
Performances of the analytical method for the determination of T-2 and HT-2 toxins in cereals

<table>
<thead>
<tr>
<th>Applicability</th>
<th>wheat (pasta), corn, barley</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection limit (signal/noise = 3:1)</td>
<td>5 ng/g (T-2)</td>
</tr>
<tr>
<td></td>
<td>3 ng/g (HT-2)</td>
</tr>
<tr>
<td>Antibody cross-reactivity</td>
<td>100% (T-2 and HT-2)</td>
</tr>
<tr>
<td>Range of applicability</td>
<td>0.01-1.4 µg/g</td>
</tr>
<tr>
<td>Recovery (spiking range 25-500 ng/g)</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>Relative Standard Deviation</td>
<td>&lt; 8%</td>
</tr>
</tbody>
</table>

Visconti et al., 2005
# Occurrence of T-2 + HT-2 toxins in durum wheat in Italy

Samples collected at storage facilities through official sampling procedure (Reg. EC. 401/2006)

<table>
<thead>
<tr>
<th>T-2 + HT-2*</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of analyzed samples</td>
<td>136</td>
<td>92</td>
</tr>
<tr>
<td>Number of samples &lt; LOD (&lt;3 ng/g)</td>
<td>55 (40%)</td>
<td>38 (41%)</td>
</tr>
<tr>
<td>Number of positive samples</td>
<td>81 (60%)</td>
<td>54 (59%)</td>
</tr>
<tr>
<td>* of which:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min (ng/g)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Max (ng/g)</td>
<td>122</td>
<td>108</td>
</tr>
<tr>
<td>Media (ng/g)</td>
<td>27,3</td>
<td>24,8</td>
</tr>
</tbody>
</table>

* HPLC/FD determination

2008 = North (51), Center (46), South (39)
2009 = North (34); Center (39), South (19)
# Occurrence of T-2 + HT-2 toxins in durum wheat in Italy

<table>
<thead>
<tr>
<th>Crop</th>
<th>Analyzed samples (n)</th>
<th>Samples distribution (n)</th>
<th>Mean of positives (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt; 3* ng/g</td>
<td>3-50 ng/g</td>
</tr>
<tr>
<td>2008</td>
<td>136</td>
<td>55 40%</td>
<td>66 49%</td>
</tr>
<tr>
<td>2009</td>
<td>92</td>
<td>38 41%</td>
<td>50 55%</td>
</tr>
</tbody>
</table>

* LOD: T-2 = 5 ng/g; HT-2 = 3 ng/g
## Confirmation by LC-MS/MS
(20% analyzed durum wheat samples)

<table>
<thead>
<tr>
<th>Sample</th>
<th>T-2 (ng/g)</th>
<th>HT-2 (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC/MS-MS*</td>
<td>HPLC/FD**</td>
</tr>
<tr>
<td>12 EP</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>22</td>
<td>36</td>
<td>16</td>
</tr>
<tr>
<td>16 EP</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>13 EP</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>GR 415</td>
<td>1</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>45 EP</td>
<td>1</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>GR 412</td>
<td>4</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>5S</td>
<td>4</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>15 EP</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>56</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>50</td>
<td>41</td>
<td>16</td>
</tr>
<tr>
<td>30</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>8</td>
<td>&lt; LOD</td>
<td>&lt; LOD</td>
</tr>
<tr>
<td>16</td>
<td>48</td>
<td>20</td>
</tr>
<tr>
<td>32</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

* LOD (LC/MS-MS): T-2 0.5 ng/g; HT-2 0.7 ng/g
** LOD (LC/FD): T-2 3 ng/g; HT-2 5 ng/g
Occurrence of T-2+HT-2 toxins in pasta

2008
Analyzed samples: 106
- 5 “mix cereals” pasta
- 13 “whole wheat” pasta
- 88 “semolina” pasta

2009
Analyzed samples: 26
- 26 “semolina” pasta

All samples <LOD
(<3 ng/g T-2; <5 ng/g HT-2)
CONCLUSIONS

- T-2 + HT-2 presence was found in Italian *durum wheat* production;

- T-2 + HT-2 content in *durum wheat* is quite low: more than 90% of positive samples are not detectable or *lower than 50 ng/gr* (mean about 25 ng/gr)

- No presence of T-2 + HT-2 has been found in *commercial pasta*: T-2 + HT-2 <LOD for all the 132 samples analyzed.
Recommendations

• Due to the **low presence of T-2 + HT-2** mycotoxins in commercial durum wheat and **very high reduction rate** occurring during cleaning and milling process, maximum limits for these mycotoxins on durum wheat does not appear necessary;

• Moreover the further reduction recorded during pasta process and the absence of T-2+ HT-2 in commercial pasta samples suggest that this product do not contribute to consumer exposure and therefore no limits are need on this product.
Acknowledgments

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The Italian Food Company, Since 1877.
Barilla G.&R. F.lli S.p.A.
Parma Italy

SECTION B: Presence of T-2 and HT-2 toxin in cereals and cereal products